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An investigation of the correlation of vitamin D status and management outcomes in patients with severe COVID-19 at a South African tertiary hospital

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Highlights

- VitD inadequacy and its prevalence in patients with severe COVID-19 admitted to ICU.
- No statistically significant relationship between VitD status and COVID-19 mortality.
- Factor associated with VitD levels and mortality included serum creatinine.
- Baseline VitD and COVID-19 patients with co-morbidities admitted in the ICU.

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ABSTRACT

Background: Severe COVID-19 has a poor prognosis, and biomarkers may predict disease severity. This study aimed to assess the effect of baseline Vitamin D (VitD) inadequacy on outcome of patients with severe COVID-19 admitted to intensive care unit (ICU) in a tertiary hospital in South Africa.

Methods: Patients with confirmed SARS-CoV-2 were recruited during wave II of the pandemic in Cape Town. Eighty-six patients were included in the study. They were categorized into three groups “VitD deficient, VitD insufficient and VitD sufficient”. We combined the VitD deficient with insufficient group to form “VitD inadequate’’ group. Cox regression analysis was done to assess the association between VitD status and mortality. Factors with p< 0.05 in adjusted multivariable cox regression were considered statistically significant.

Results: The proportion of VitD inadequacy was 64% (55/86), with significantly higher proportion of hypertension (66%; p 0.012). Kaplan Meir curve showed no significant difference in the probability of survival among the COVID-19 patients admitted in the ICU with or without VitD inadequacy. However, patients with elevated serum creatinine were significantly more at risk of dying (Adjusted Hazard Ratio 1.008 (1.002 – 1.030, p<0.017).

Conclusion: Our study found a high prevalence of VitD inadequacy (combined deficiency and insufficiency) in COVID-19 patients admitted to the ICU. This may indicate a possible risk of severe disease. Whilst there was no statistically significant relationship between VitD status and mortality in this cohort, baseline VitD may be an important prognostic biomarker in COVID-19 patients admitted to the ICU, particularly in those with comorbidities that predispose to VitD deficiency.

Keywords: COVID-19, prognosis, ICU, Vitamin D, outcomes.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) quickly spread across the world leading to a global pandemic and early waves had a very high mortality rate among severe cases. As of 14 May 2023, globally, there have been 766 million confirmed cases of COVID-19, including 6.9 million deaths, reported to the WHO[1]. Patients with severe COVID-19 developed what has now been termed a 'cytokine storm' associated with severe acute respiratory syndrome (SARS) and multi-organ failure[2]. This cytokine storm, a hyper-inflammatory syndrome driven by activation of the immune system, is associated with the production of very high levels of pro-inflammatory cytokines and other inflammatory markers which are released into the systemic circulation[3,4].

Vitamin D (VitD) is known to influence immune system regulatory function[5,6].

Most of circulating VitD (90%) is produced by cutaneous conversion of 7-dehydrocholesterol by ultraviolet light to form the precursor, VitD3, and the rest is obtained from the diet (VitD2); both forms are hydroxylated in the liver to form 25-hydroxy VitD3 and VitD2, respectively[7]. The active form of the vitamin is synthesized by a second hydroxylation in the kidney by the 1-alpha hydroxylase enzyme to form the active metabolite called 1,25(OH)2 D (1,25-dihydroxy vitamin D or calcitriol)[8]. This enzyme is also present in immune cells such as macrophages and monocytes, where its activity is under the control of cytokines such as interleukins (IL) 1,2 and 15, tumour necrosis factor alpha and interferon-gamma[9]. The downstream effect of VitD is the upregulation of genes required for the synthesis of substances such as defensins and cathelicidin involved in the immune function[10–13]. The significant role of active VitD as a selective immunosuppressant

is illustrated by its ability to either prevent or markedly suppress animal models of autoimmune disease[10].

Other studies have found that VitD can also prevent or markedly suppress experimental autoimmune encephalomyelitis, Guillain-Barre syndrome, rheumatoid arthritis, systemic lupus erythematosus, type I diabetes, and inflammatory bowel disease[14,15]. It was postulated that this may also be the case in severe COVID-19, where the marked cytokine inflammatory reaction may be dampened by VitD[16].

In South Africa, healthy adults are reportedly VitD-sufficient, however, the majority of those aged above 65 years are deficient[17]. In this population, VitD deficiency and insufficiency were present in 27% and 38%, respectively[17]. It is unclear how VitD deficiency and insufficiency affect COVID-19 outcomes in the intensive care unit (ICU). This study aimed to assess the effect of VitD deficiency and insufficiency on patients with severe COVID-19 admitted to the ICU in a South African tertiary hospital.

MATERIALS AND METHODS

Study population

This was a prospective cohort study of patients admitted to the ICU at Tygerberg Hospital during the second wave from 29 October 2020 to 10 February 2021. A total of 86 patients were followed up until a definitive outcome of either died or discharged alive from ICU to a step-down ward was established. These patients had confirmed COVID-19 by a polymerase chain reaction (PCR) assay for the causative severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

The study only included adults (≥18 years). ICU admission was based on disease severity and availability of critical care resources. Admission serum samples were

obtained and stored at -80°C. Figure I, shows the process followed to obtain the final cohort for VitD testing.

[Insert Figure I. Study flow chart]

Study setting

Tygerberg Hospital is a 1380 bed tertiary care hospital, located in the northern suburbs of the Cape Town metropole, Western Cape, in South Africa. The hospital serves a community of approximately 3.6 million from the public health system and is the main teaching hospital for Stellenbosch University Faculty of Medicine and Health Sciences.

Data collection

Clinical and demographic data were prospectively collected from inpatient medical records. Due to COVID-19 restrictions and to limit disease spread, pictures of patient file notes were obtained at admission for detailed perusal and data capturing, outside of the wards. This was achieved by the transfer of hand-written notes into the REDCap electronic database through pictures of the files which contained all clinical information and ICU admission notes. Laboratory data was obtained from the hospital laboratory information system (TrakCare Lab Enterprise). The laboratory service at Tygerberg Hospital is provided by the National Laboratory Health Service (NHLS) which serves all public hospitals in South Africa. Validity and quality checking of the data was performed by the supervisor of the data entry process. VitD status of participants was categorised into deficiency when <30 nmol/L (<20 ng/ml), insufficiency when between 31 – 50 nmol/L (21–29 ng/ml) and sufficiency if above 50 nmol/L (30 ng/ml) according to the Endocrine Society guidelines[18]. The body mass

index (BMI) could not be assessed due to difficulties in accurately measuring the weight and height of critically ill patients on a hospital bed.

Laboratory Analyses

Serum and whole blood samples were obtained on admission to the ICU for each patient. The serum tubes were centrifuged after they were adequately clotted, and one millilitre was aliquoted into cryotubes and stored at -80 °C until analysis could be performed. The tubes and storage places were marked with unique patient identification numbers for easy retrieval. The following biochemical laboratory tests were performed: urea, creatinine, calcium (Ca), phosphate (P), magnesium (Mg), VitD, troponin T (TropT) and N-terminal pro-brain natriuretic peptide (NT-proBNP).

The D-dimer was performed immediately after collection of the samples on whole blood, using the Sysmex CS-2000i™ (Sysmex Medical Electronics, Germany). VitD levels were determined using the Roche Cobas 6000 Elecsys II (Roche Diagnostics, Mannheim, Germany) total VitD assay. This assay uses the electrochemiluminescent method to detect both VitD2 and VitD3. The analytical methods of the other biochemistry tests are described in an earlier study[19]. The estimated glomerular filtration rate (eGFR) was obtained with all serum creatinine results from the laboratory, using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula without a correction for the race as recommended by a local study[20]. Strict internal quality control procedures were followed, and the laboratory participates in external quality assurance schemes, is accredited by the South African Accreditation Service (SANAS) and complies with ISO15189 regulations.

Ethics

Research approval was obtained from the Health Research Ethics Committee of Stellenbosch University (approval number N20/04/002_COVID-19). A waiver of consent was obtained from the Ethics Committee due to the expedited nature of this study. The research project was conducted according to the ethical principles of the Declaration of Helsinki. Patient data was anonymized to protect their privacy and adhere to the Protection of Personal Information Act of South Africa[21].

Outcomes and predictor variables

The main outcome of interest was mortality while in ICU with VitD as the main exposure. The patients were categorised into three groups: VitD deficient (<30 nmol/L), insufficient (30 – 50 nmol/L and sufficient (>50 nmol/L). As only 8% of cases were VitD deficient, this group was combined with the insufficient group to form a variable called 'VitD inadequate' (<50 nmol/L). Comparative analyses were undertaken between the VitD inadequate and the VitD sufficient groups to assess differences and similarities in relation to mortality among the patients admitted to ICU. Patients were stratified by VitD status and the proportion of those who died in ICU. The clinical variables studied included the need for respiratory support and mortality stratified by the VitD status.

Previous medical history was documented including co-morbidities such as hypertension, diabetes mellitus, dyslipidaemia and smoking status.

STATISTICAL ANALYSES

Continuous variables were expressed as median with inter-quartile range since they were non-normal data. Categorical variables were expressed using frequencies and percentages. Chi-squared and Fisher exact test were used to assess the association between VitD status and the categorical variables. Median test was used to assess the equality of the median of the continuous variables between the VitD status groups. Schoenfeld residuals and the Cox proportional hazards test were used to assess the proportional hazards assumption. Cox regression was used to assess significant association between demographic, VitD status, and mortality. Factors associated with mortality and VitD status at $p< 0.05$ in unadjusted univariable cox regression were included in a multivariable model to identify predictor variables associated with VitD status and mortality. Adjusted hazard ratios and their 95% confidence intervals (CIs) were used as a measure of association. All statistical analyses were performed using Stata (V.16, Stata Corp, College Station, Texas, USA) and R (V, 4.1.0, R Core Team) with R Studio (V.1.4.1, R Studio Team) statistical software.

RESULTS

Of the total of 86 patients included in the study and grouped by their VitD status; 71% were females (n = 61). Because of the skewed sex distribution, both the inadequate and sufficient groups were dominated by females at 69% and 74%, respectively.

Most patients in the inadequate group had diabetes (53%, n=25, p = 0.670) and hypertension (66%, n=31, p = 0.012) (Table 1).

Insert Table 1

Both groups had elevated inflammatory markers such as CRP and ferritin. The ferritin levels were, however, lower in the inadequate group, although still above the reference interval. The rest of the biochemical markers did not show any differences between the two groups (Table 2). Factors that were found to have an association with VitD levels and mortality included sex and creatinine with an unadjusted hazard ratio (HR) of 1.66 (1.00 – 2.75) for sex (p= 0.05) and an adjusted HR of 1.008 (1.002 – 1.03) for serum creatinine (p = 0.017) (Table 3). Patients with VitD inadequacy had a higher death rate compared to the sufficient group, 64% and 59% respectively.

Insert Table 2. Biochemical results.

A slightly lower proportion of those with VitD inadequacy required invasive ventilation, while the sufficient group had a slightly higher proportion of patients requiring invasive ventilation (41% vs 38%).

Patients in the inadequacy group had a higher mortality (64%) as compared to the sufficient group (59%). The rate of ICU admission appeared to be higher in patients with VitD levels > 50 nmol/l vs VitD = 50 nmol/l on Day 10 (73% vs 70%), and 27% vs 25% on Day 30. (Figure 2). The survival curve HR, on the other hand, was not statistically significant, with HR: 0.99 (95% CI: 0.61-1.62) and p = 0.65. (Figure 2).

[Insert Figure 2.]

[Insert Table 3]

The proportional hazards assumption was tested using plots of the scaled Schoenfeld residuals of each covariate against log-time. The Schoenfeld residuals test revealed that a proportional hazard assumption had the same effect on COVID-

19 patients' survival rates with VitD = 50 nmol/l and VitD > 50 nmol/l (p = 0.202) (Figure 3).

[Insert Figure 3]

DISCUSSION

This study investigated the VitD status in a cohort of patients admitted to ICU at Tygerberg Hospital and determined whether VitD status had any correlation with outcomes. Most patients were middle-aged with a female preponderance. The main comorbidities identified were hypertension and diabetes. We found that most of the patients had VitD inadequacy; only a very small percentage had VitD deficiency, and this group was too small to perform any further statistical analysis on. Patients with hypertension and diabetes mellitus had a higher prevalence of VitD inadequacy.

The mortality rate was higher in the group with inadequate VitD levels. However, these differences in mortality were not statistically significant. The proportion of patients requiring invasive ventilation was slightly higher in the sufficient VitD group. While this finding may show no statistical significance, physiologically, it could be explained by presence of the cytokine storm which causes ARDS and may override the modulatory effect of effectors including VitD and cytokines on the immune system[22]. In addition, we could postulate that VitD inadequacy may have a ‘’protective” effect from the cytokine storm i.e. they have a blunted immune response, especially in the lungs, so that even with severe disease complicated by multi-organ failure, their lung function may remain slightly preserved compared to those with adequate VitD and a hyper-inflammatory response.

We found no statistically significant association between VitD status and inflammatory markers, liver function and myocardial injury markers. The finding of lower serum ferritin levels in the VitD inadequate group may be explained by several factors involved in immune system regulation and its role in iron storage[23]. The levels of VDBP have been reported to be low during acute inflammatory illness, making VDBP a negative acute phase reactant[24]. Some authors have further suggested that total VitD measurement is unreliable in the acutely ill patient because it will be falsely low due to reduction of both VDBP and albumin which transport VitD in circulation[25]. However, other researchers have found normal or elevated VDBP levels in patients with inflammatory disorders. Aksan et al reported a positive correlation between high sensitivity CRP and VDBP, and concluded that total serum VitD is still a reliable marker of the VitD status in this group of patients[26].

Only serum creatinine showed a statistically significant association with mortality; however, this difference is not clinically significant as both groups had serum creatinine concentrations within the reference interval. The current study's VitD status findings (68% VitD inadequacy) are consistent with those of De Smet et al, who described a baseline prevalence of VitD inadequacy of 67% in patients with COVID-19, using a cut-off of 50 nmol/L[27]. A South African study found the prevalence of VitD inadequacy to the 82%, the majority of which were VitD deficient at 66%, with only 8% having sufficient VitD levels. Their cohort included one hundred patients with symptomatic but variable severity of COVID-19[28]. In contrast to the South African findings from ours and the above study, Teama et al found a very high prevalence of VitD inadequacy in those with severe COVID-19 in an Ethiopian hospital[13]. Their study found a prevalence of VitD inadequacy of 97.6% and calculated a cut-off level of <45 nmol/L to be predictive of poor prognosis, with a

sensitivity of 75.9% and specificity of 60.6%. Our cohort was slightly older at admission with co-morbidities such as hypertension and diabetes more prevalent compared to a previous study by Yoo et al[29]. Another study also reported that the above co-morbidities have been associated with VitD deficiency[30] and a higher risk of severe COVID-19[31, 32].

It is now common knowledge that the SARS CoV-2 virus binds to the Angiotensin-converting enzyme 2 (ACE2) receptors, leading to upregulation of the classic arm of the Renin-Angiotensin-Aldosterone System(RAAS) pathway[33]. The effects of the classic RAAS pathway include vasoconstriction, oxidative stress, pro-inflammatory state, aldosterone release, cell proliferation and tissue fibrosis[34]. Patients with VitD deficiency also have increased RAAS activity and angiotensin II levels which potentially causes the aforementioned physiological changes[35]. Furthermore, VitD reduces renin expression and action against any regulatory feedback by angiotensin II, while the opposite is true in VitD deficiency which shows an increase in renin expression[36]. The increased renin activity in VitD deficiency leads to up-regulation of ACE2 activity which in turn increases conversion of angiotensin I to Ang II; the latter has been postulated to be toxic to the lungs through its action on Ang II type I receptors causing severe inflammation and lung fibrosis[37]. Plasma renin is also elevated in those with hypertension, diabetes mellitus and in most elderly patients[38]. This common thread of hyper-reninaemia between these conditions and VitD inadequacy may possibly be one of the underlying physiological mechanisms leading to the high risk for severe COVID-19 seen in patients with these co-morbidities.

An association of VitD deficiency and low eGFR associated with an increased level of IL6 as a risk of SARS-CoV-2 infection progression and a poor prognosis has been reported[39]. In addition, a low VitD level is associated with elevated pro-inflammatory cytokines and was demonstrated to be an independent predictor of COVID-19 severity[40]. This association was also demonstrated in an Egyptian cohort in which serum VitD was found to be inversely related to inflammatory markers such as ferritin in COVID-19 patients[41]. Other researchers found serum VitD levels to be negatively correlated with ferritin among COVID-19 patients admitted to the ICU[13]. Our findings of low ferritin levels in those with VitD inadequacy are unexpected and in direct contrast of this existing data. Physiologically, this could mean that in some people, VitD inadequacy predisposes to a limited immune response with less elevation of inflammatory markers such as ferritin. However, ferritin was raised above the upper limit of the reference interval in both groups, and the apparent difference between the two groups did not show statistical significance.

Our study results add to the growing body of observations that low VitD levels are associated with developing more severe COVID-19. Several studies have published findings and recommendations on VitD supplementation for high-risk population groups of patients with COVID-19. A recent systematic review and meta-analysis found that VitD supplementation may be beneficial for clinical outcomes of COVID-19, especially when treatment begins following the diagnosis[42]. Another systematic review showed that VitD supplementation was protective against severe COVID-19 and recommended that it can be used as an adjunct to other medical management measures[43], while a narrative review by Bae et al concluded that VitD

supplementation to maintain serum levels above 50 nmol/L can be beneficial in managing the risk and possibly mitigate the mortality associated with severe COVID-19[44].

However, more recent clinical trials found that VitD supplementation had no effect on the risk of SARS CoV2 infection, disease development or the severity thereof[45, 46]. Additionally, there was some controversy on the role of VitD in COVID-19 after some earlier studies were retracted due to the fast tracking of publications early in the pandemic[47, 48]. In our view, even if the current data on VitD supplementation may be inconclusive, it remains important to further investigate any factors that may be protective against severe COVID-19.

South Africa has a high burden of infectious diseases including human immunodeficiency virus (HIV) (8.23 million people) and tuberculosis (TB), with a prevalence of 737 per 100 000 population[49]. The SA National Institute for Communicable Diseases (NICD) also reported that TB is the leading cause of death in South Africa, accounting for 6% of all deaths; HIV is the fifth commonest cause of death in South Africa with a co-infection rate between 28.8% and 59%[50]. Some studies found an association between inadequate VitD status and the development of TB and other adverse outcomes[51, 52]. Lung damage from previous or current TB infection may predispose these patients to acute respiratory distress syndrome (ARDS) and possibly respiratory failure due to the characteristic lung-centric inflammation found in severe COVID-19[53].

HIV and anti-retroviral therapy have been shown to affect VitD metabolism, mostly affecting bone health[54]; the immune system may also be affected and requires further studies to investigate. Another South African study found that VitD deficiency

was as prevalent as 42%–74% among HIV infected individuals[55]. It is therefore reasonable to postulate that VitD supplementation could benefit this group of patients as well, and needs further investigation in future studies. Although we have no data on these two infectious diseases in our cohort, it is possible that they may play a role in the course of COVID-19 disease especially in those who are undiagnosed, with an unsuppressed viral load or low CD4 cell count that may affect their morbidity and mortality from COVID-19. There is paucity of data on VitD status and its relationship to COVID-19 severity and outcome from the African Continent. This study therefore creates an opportunity to further interrogate the role of VitD in viral infections with a focus on ascertaining its relationship to severity of diseases caused by SARS-CoV2. This study adds value due to the homogenous nature of the patient population; all were admitted in the ICU, managed by the same group of clinicians, and all the VitD tests were performed on the same day by the same laboratory thereby avoiding the effects of intra-laboratory variability on patient results. To our knowledge, this is among the few studies done assessing the effect of VitD on COVID-19 severity and outcome from an African population. There are two important limitations of the study to bear in mind. One is the small sample size of both the whole cohort and the VitD deficiency group. This might have diluted the effect of VitD deficiency on disease outcome. The second main limitation is the lack of a control group of patients with mild or moderate disease; this limited any comparison of VitD status and outcomes by disease severity. Larger, multi-centre studies in Africa are needed that would increase the power of the study to detect meaningful differences.

CONCLUSION

Our study found a high prevalence of VitD inadequacy- combination of deficiency and insufficiency- in ICU admitted COVID-19 patients during the second wave; this may have contributed to their risk of severe disease. Even though there was no statistically significant relationship between VitD status and mortality in this cohort; this study provides evidence that baseline VitD may play an important role in these patients, particularly those with comorbidities that are associated with VitD inadequacy.

FOOTNOTES

Author Contributions: Conceptualization: TPJ, AEZ Study design: LNS, AY, PSN, data collection: TPJ, VDN, AEZ; statistical analysis: LNS, AY, PNS; drafting the manuscript: TPJ, LNS, AEZ, PSN. Reviewing manuscript for intellectual content MJK, TME and RTE. Reviewing final drafting of the manuscript: TPJ, LNS, MJK, AY, VDN, JTL, ZCC, BWA, CFK, EMI, UL, TEM, RTE, AZ, AEZ, PSN. All authors approved the last version of the manuscript.

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Informed Consent Statement: The Investigators obtained ethical approval and waiver of consent from the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Stellenbosch University and Research Committee of the Tygerberg Hospital Ethics approval number N20/04/002_COVID-19.

Conflicts of Interest: No conflict of interest declared

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Table 1: Socio-demographic information and biochemical profiles according to Vitamin D status.

Characteristic		**Vitamin D categories**		
	Total (n=86)	**<=50 nmol (n=55)**	**> 50 nmol (n=31)**	**p-value**
Age at admission	54.33 (46.10-62.00)	55.99 (47.20-63.00)	53.00 (39.89-60.13)	0.093
Gender				0.660
Female	61 (71%)	41 (69%)	20 (74%)	
Male	25 (29%)	18 (31%)	7 (26%)	
Hypertension				0.012
Yes	38 (56%)	31 (66%)	7 (33%)	
No	30 (44%)	16 (34%)	14 (67%)	
Hyperlipidaemia				0.640
Yes	5 (7%)	3 (6%)	2 (10%)	
No	63 (93%)	44 (94%)	19 (90%)	
Diabetes				0.670
Yes	35 (51%)	25 (53%)	10 (48%)	
No	33 (49%)	22 (47%)	11 (52%)	
Death/Discharge				0.650
Discharge	32 (37%)	21 (36%)	11 (41%)	
Death	54 (63%)	38 (64%)	16 (59%)	
Ventilation				0.772

Non-invasive	49 (57%)	32 (62%)	16 (59%)
Invasive	37 (43%)	20 (38%)	11 (41%)

Table 2. Biochemical results.

Abbreviations: $Ca^{2+:}$ calcium, CRP: C-reactive protein, cTropT: cardiac Troponin T, eGFR: estimated glomerular filtration rate, HbA1c: Glycated haemoglobin, $Mg^{2+:}$ Magnesium, NTProBNp: N-terminal pro B-type natriuretic peptide, PCT: Procalcitonin.

			Vitamin D categories		
	Reference Interval	**Total (n=86)**	**<=50 nmol (n=55)**	**> 50 nmol (n=31)**	*p-value*
D Dimer (n=71)	0.00-0.25 mg/L	0.78 (0.40-3.50)	0.77 (0.35-3.75)	0.82 (0.47-1.51)	0.970
cTropT (n=63)	< 100 ng/L	13 (6 - 27)	13 (7 - 27)	14 (5 - 27)	0.950
LDH (n=34)	100–190 U/L	736 (582 - 900)	696 (602-891)	818 (542-980)	0.620
NTproBNP (n=59)	< 125 ng/L	179 (88 -791)	174 (105-642)	188 (68-1587)	0.950
HbA1c (n=70)	< 6.5 %	7.6 (6.3 - 8.0)	7.60 (6.3 - 9.0)	7.4 (6.1 - 8.6)	0.540
PCT (n=61)	< 0.5 µg/L	0.31 (0.15-1.12)	0.35 (0.16-0.96)	0.30 (0.14-1.38)	0.990
Ferritin (n=50)	13–150 μg/l	855 (469 -1292)	721 (469 -1088)	1055 (434 -2561)	0.310
CRP (n=77)	< 10 mg/L	137 (86 - 221)	137(80 - 224)	139 (107 -221)	0.750
Ca^{2+} (n=46)	2.12–2.59 mmol/l	2.06 (2.01-2.18)	2.05 (2.01-2.19)	2.09 (2.02-2.16)	0.960
Mg^{2+} (n=46)	0.63–1.05 mmol/l	0.94 (0.89-1.06)	0.97 (0.89-1.07)	0.93 (0.82-1.01)	0.120
Phos (n=36)	0.78–1.42 mmol/l	1.15 (0.93-1.53)	1.17 (0.91-1.57)	1.12 (1. 00-1.35)	0.580
Urea (n=79)	2.1–7.1 mmol/l	6.7 (4.9 - 8.6)	6.9 (5.1- 8.7)	5.8 (4.4 - 7.9)	0.160
Creatinine (n=79)	49–90 µmol/l	76 (66 - 102)	76 (65 - 101)	76.00 (69 - 106)	0.600
eGFR (n=77)	> 60	80 (70 - 96)	80 (70 - 100)	80 (72 - 92)	0.760

Table 3: Factors associated with mortality, vitamin D status and demographics

Characteristic	**HR (95% CI)**	**p-value**	**AHR (95% CI)**	**p-value**
Vitamin D	1.00 (0.99-1.01)	0.932	1.001 (0.99-1.02)	0.883
Age at admission	0.99 (0.97-1.01)	0.424	0.98 (0.96-1.01)	0.159
Gender: Male	1.66 (1.00-2.75)	0.05	1.39 (0.80-2.44)	0.245
Hypertension	1.08 (0.63-1.87)	0.779	1.19 (0.61-2.33)	0.608
Diabetes	1.13 (0.69-1.85)	0.627	1.02 (0.55-1.92)	0.932
Ventilation: invasive	1.35 (0.85-2.17)	0.203		

Ddimer	1.02 (0.98-1.08)	0.234		
NTProBNp	1.000 (0.99-1.00)	0.667		
HbA1c	1.03 (0.95-1.11)	0.536		
Creatinine	1.006 (1.0003-1.01)	0.039	1.008 (1.002-1.03)	0.017
CRP	0.999 (0.996-1.00)	0.311		
Ca^{2+}	0.41 (0.04-3.84)	0.438		
eGFR	0.99 (0.98-1.00)	0.119		
Urea	1.01 (0.96-1.07)	0.602		
$Mg2^{+}$	0.84 (0.15-4.78)	0.846		
Ferritin	1.00 (0.99-1.0002)	0.997		
Phosphate	2.46 (1.23-4.90)	0.01		
PCT	1.17 (0.91-1.52)	0.208		

Abbreviations: HbA1c: Glycated haemoglobin, NTProBNp: N-terminal pro B-type natriuretic peptide, CRP: C-reactive protein, $Ca^{2+:}$: Calcium, eGFR: estimated glomerular filtration rate, Mg^{2+}: Magnesium, PCT: procalcitonin.

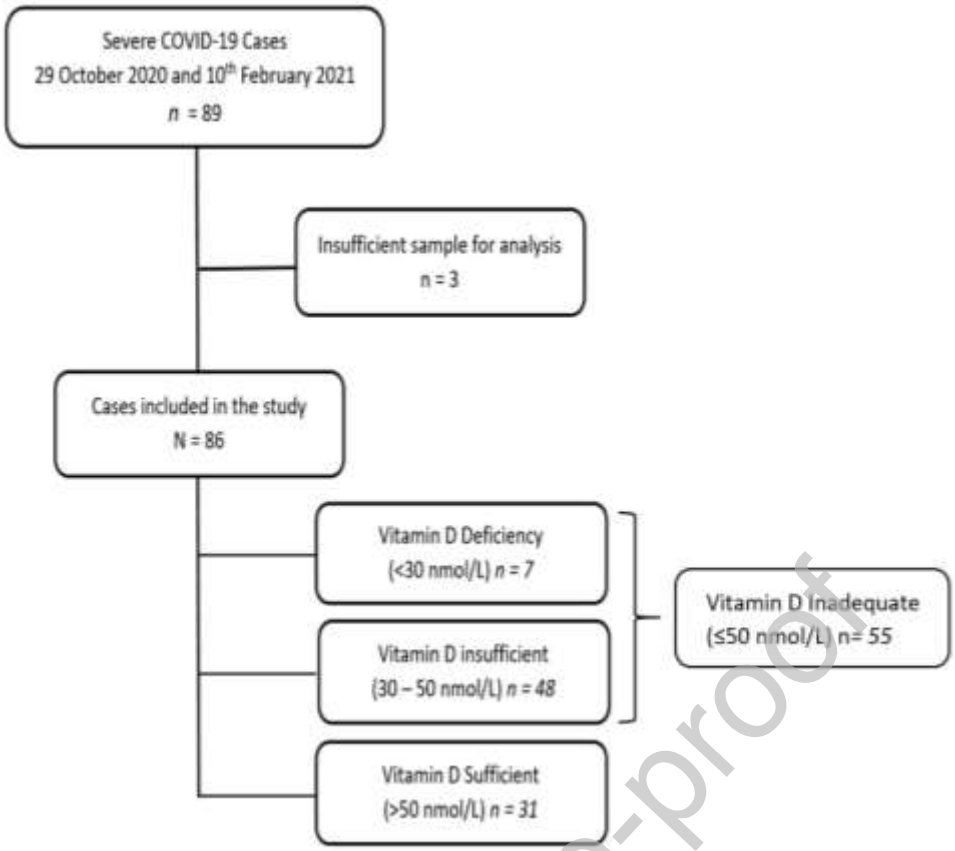

Figure I. Study flow chart.

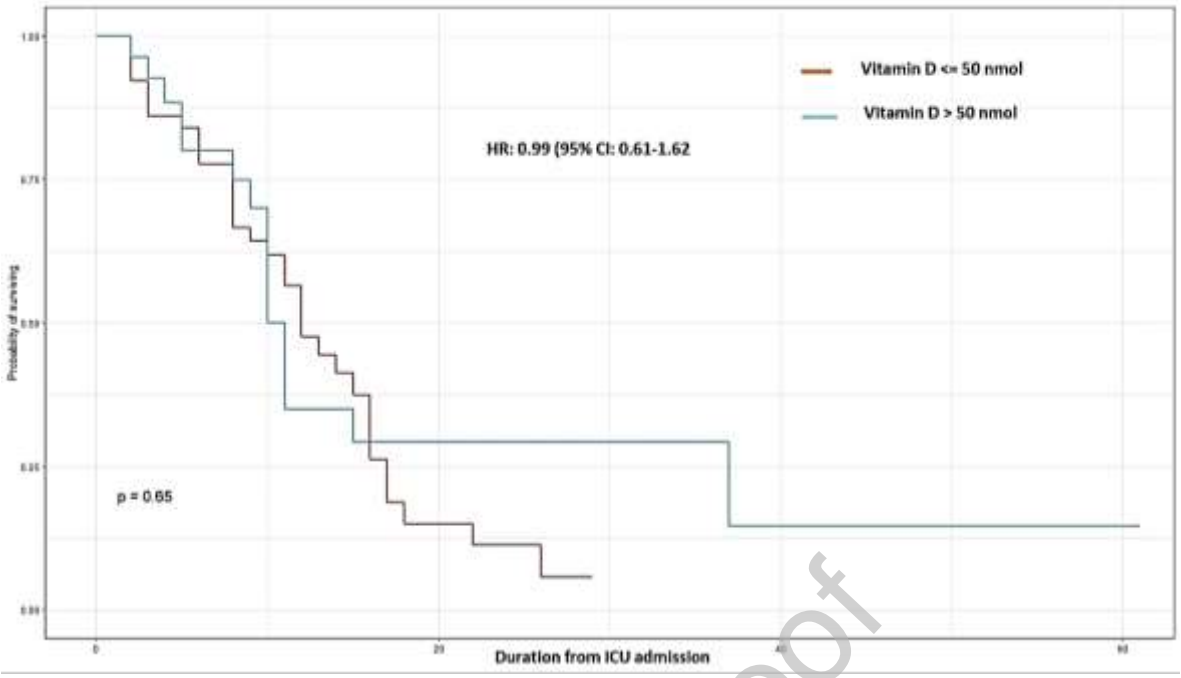

Figure 2: Probability of survival curve of COVID-19 patients admitted in the ICU stratified by vitamin D status.

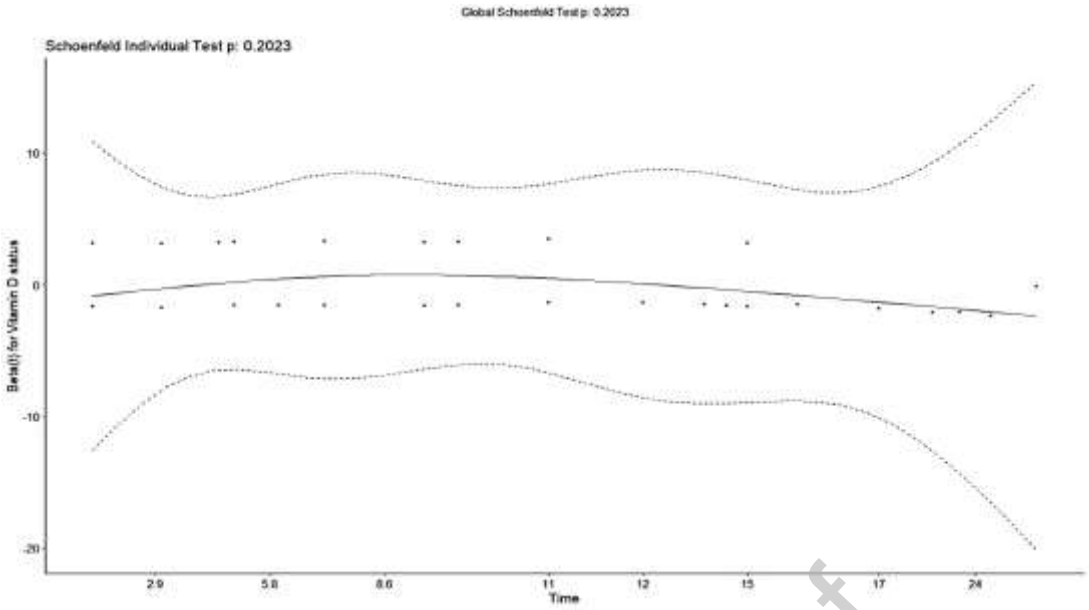

Figure 3: Assessing proportional hazards function using Schoenfeld residuals.

Conflicts of Interest: all authors declare no conflicts of interest.